Theory and Phenomenology of Mixed Amphiphilic Aggregates

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Abstract

We give a short overview of existing approaches describing shapes and energetics of amphiphilic aggregates. In particular, we consider recent experimental data and theory in relation to *mixed* aggregates. We point out the outstanding questions deserving further investigations such as stability of single-component vesicles and size growth of mixed vesicles induced by micelle-forming surfactants.

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1 Introduction

An amphiphilic molecule consists of two moieties with opposing properties: hydrophilic polar head and hydrophobic hydrocarbon tail. Such hydrophilic-hydrophobic nature of amphiphiles determines their self-assembly and fascinating physical properties. Numerous investigations have recently addressed structures and phase behavior of amphiphiles in aqueous solutions [1]. This interest is partially motivated by the significant role played by amphiphiles in biophysics, biotechnology and pharmacology [2, 3]. Indeed, an important class of amphiphiles, called phospholipids, form the basis of cell membranes, while various surfactants such as octyl glucoside (OG) and bile salts are used for extraction of proteins from cell membranes (membrane solubilization) and for preparation of artificial phospholipid membranes containing specific proteins (membrane reconstitution).

Self-assembly in water has many features in common for different amphiphiles. First, as the amphiphile concentration becomes higher than a critical one, the amphiphilic molecules assemble into monomolecular layers (monolayers), which are about $\delta \simeq 1.5$nm thick and have one surface covered by the hydrophilic heads and the second formed by the hydrophobic tails. Then, these monolayers self-organize into structures where all the hydrophobic surfaces are shielded from contact with water by the hydrophilic ones.

The difference between aggregates formed by various amphiphiles is in the shapes of the monolayers [1, 4, 5, 6, 7]. We describe the shape of a monolayer by its characteristic curvature c or radius of curvature $R = 1/c$. A more exact definition will be given below. The monolayers of many common phospholipids such as lecithin have nearly flat geometries so that their radii of curvature are much larger than the monolayer thickness, $R >> \delta$. In water they form flat bilayers organized as multi-lamellae or unilamellar vesicles [2]. The latter (so-called liposomes for phospholipids) are small "bags" made of amphiphilic bilayers of dimensions in the range of few nm to several μm. Another type of amphiphiles, called detergents (micelle-forming surfactants), forms in many cases monolayers which are strongly curved away from the aqueous surrounding with a radius of curvature of the order of the monolayer thickness, $R \simeq \delta$. Due to their high curvature such monolayers exist in water in the form of cylindrical or spherical micelles.

Besides flat bilayers and micelles, which are the main subject of this presentation, few other characteristic shapes are observed for amphiphile monolayers. One of them is also characterized by fairly high curvatures opposite to the curvature of micelle-forming surfactants (directed away from the hydrophobic tails). Such amphiphiles (for example, dioleoylphosphatidylethanolamine (DOPE)) form in water an inverted hexagonal phase (so-called the H_{II}) at high enough concentrations [5, 7]. Surfactants of another type (*e.g.*, monoolein) tend to form monolayers of saddle-like shape. The corresponding structures in water are referred to as bicontinuous cubic phases [6].

While the shapes of monolayers formed by individual amphiphiles are known in many cases, they are less well understood in the case of monolayers consisting of mixtures of different amphiphiles. In particular, mixtures of bilayer-forming lipids and micelle-forming surfactants are of importance to membrane solubilization and reconstitution, as has been recently investigated by several experimental groups. Since detailed reviews of this phenomenology exist [4, 8], we give here only a brief summary of the results.

- The unilamellar liposomes in a single-component lipid (lecithin) retain their shape and size for long times following their preparation.

- Upon addition of small amounts of micelle-forming surfactant to the lipid vesicles, the system stays in the form of stable mixed vesicles. However, the vesicle size changes as a function of concentration of the added surfactant. In most cases vesicle growth is observed and cannot be explained only by the addition of the surfactant material. The increase of the vesicle size can be associated with processes such as fusion and material transport mediated by the micelles. However, in recent studies of solubilization of vesicles consisting of dimeric amphiphiles [9], an opposite effect of a decrease in the vesicle size has been observed by cryo-transmission electron microscopy (cryo-TEM).

- When the amount of surfactant in the system reaches a critical surfactant-to-lipid ratio R_e^{SAT}, a first-order phase transition from the mixed liposomes into mixed micelles is observed.

The resulting micelles have, in most cases, a thread-like shape. However, direct formation of spheroidal micelles (and lack of thread-like micelles) has been reported for the so-called dimeric or gemini surfactants, (*e.g.,* alkanediyl-α, γ-bis (dimethyl-dodecylammonium bromide) [9]. In studies of solubilization of lecithin vesicles by a cationic surfactant cetyltrimethylammonium chloride (CTAC) a new structure intermediate between liposomes and thread-like micelles has been reported [10]. This intermediate structure consists of perforated bilayers *i.e.,* liposomes filled with holes.

The transition of liposomes into micelles progresses with increase of surfactant to lipid ratio R_e from R_e^{SAT} to a higher value called R_e^{SOL}. Inside this range the mixed liposomes seem to be in thermodynamical equilibrium with mixed micelles.

- At R_e^{SOL} only micelles are present in the aqueous solution. Further increase of the amount of surfactant results in the case of thread-like micelles in reduction of their sizes till they become spheroidal.

Variety of shapes of amphiphilic monolayers and the transitions between them pose a challenge to theoreticians. Two theoretical approaches describing such systems should be mentioned. In one detailed molecular structure and the microscopic interactions are taken into account [11]. In this review we describe a different approach to surfactant systems in terms of elastic properties of monolayers and try to point out the questions deserving further analysis.

2   Models of Membrane Elasticity

We mention several basic models describing elastic properties of amphiphilic systems. The first formulated in 1973 by Helfrich [12] determines elastic behavior of lipid membranes whose shapes only slightly deviate from a flat surface. The local shape of the membrane surface can be characterized by its principal curvatures c_1 and c_2, and its elastic energy per unit area is written as

$$f = \frac{1}{2}\kappa(c_1 + c_2 - c_0)^2 + \bar{\kappa}c_1 c_2 \qquad (1)$$

where the elastic properties of the flat membrane are determined by the bending modulus κ, the Gaussian curvature modulus $\bar{\kappa}$ and the spontaneous curvature c_0. While κ has a common meaning of rigidity with respect to changes of the curvature, c_0 and $\bar{\kappa}$ have a more delicate physical origin. The spontaneous curvature c_0 accounts for non-vanishing stress in the flat membrane ($c_1 = c_2 = 0$). In qualitative terms c_0 reflects a tendency of the flat membrane to curve spontaneously in order to relax its internal stresses. The modulus of the Gaussian curvature $\bar{\kappa}$ has a physical meaning of membrane stress related to the Gaussian curvature $c_1 \cdot c_2$. The energy determined by $\bar{\kappa}$ depends, according to the Gauss-Bonnet theorem, only on the membrane topology, and changes at processes such as fusion or fission of liposomes. The basic assumption in (1) is that the radii of curvature are much bigger than the membrane thickness, $|c_1\delta|$, $|c_2\delta| \ll 1$, so that only contributions up to second order in these small parameters are retained.

The Helfrich model (1) turned out to be very useful in analyzing the elastic and thermodynamical properties of lipid vesicles. However, the description of micelles by (1) is not straightforward because of the high curvatures existing in the micellar packing.

Another approach describing formation of amphiphilic aggregates in terms of effective molecular dimensions has been proposed by Israelachvili et al [13]. It makes use of the effective packing parameter p of an amphiphilic molecule, which combines the molecular volume v, the molecular area a and the length of hydrocarbon chain in its fully extended state l such that $p = v/(al)$. In crude terms, formation of micelles is expected for $p < 1$, molecules with $p \simeq 1$ are supposed to form flat monolayers (bilayers in water), while amphiphiles with $p > 1$ are predicted to form inverted cylinders of the H_{II} phase. Although this model explains qualitatively shapes of amphiphilic aggregates, it does not allow to calculate the energy of monolayers and the phase transitions between different structures.

An elastic model of strongly curved monolayers has been formulated by Gruner [14] for the inverted monolayers of H_{II} phases and has been later extended for monolayers of arbitrary shapes [15]. Formally, Gruner model is similar to the Helfrich one, as the energy per unit area of a cylindrical monolayer is related to its curvature by

$$f = \frac{1}{2}\kappa(c - c_{in})^2 \tag{2}$$

where κ is the bending modulus. The parameter c_{in} called the intrinsic curvature is formally analogous to the Helfrich spontaneous curvature c_0. However, it has a different physical meaning. While c_0 accounts for the bending stress in the flat state of the monolayer, c_{in} determines the monolayer shape in a completely relaxed stress-free state. The model (2) assumes that the curvature only slightly deviates from its intrinsic value so that $|(c - c_{in})/c_{in}| \ll 1$, and the bending energy is expanded up to quadratic order in this small value. On the other hand and in contrast to model (1), the curvature itself can be arbitrarily high.

The model (2) in its extended form [16] has been recently used to explain experimental results on osmotic-stress deformations of the strongly curved cylindrical monolayers of H_{II} phases and phase transitions between these monolayers and the flat bilayers of lamellar phases. In those studies it has been assumed that the quadratic expression (2) is valid also for strong bending deformations where $|(c - c_{in})/c_{in}| \simeq 1$. In other words, it was assumed that the bending stress-strain relationships are linear in a wide range of deformations. The good agreement between the predictions of the model and the experimental results, indeed, supports this assumption. On the other hand, the physical consequences of this result have still to be understood in terms of microscopical structure of monolayers.

The elastic moduli κ, $\bar{\kappa}$ and the spontaneous curvature c_0 of monolayers introduced phenomenologically in the pioneering theory (1) have been computed in detail by a mean-field approach developed in the series of works and has been reviewed recently [17]. The analysis of the monolayer material parameters in terms of the hydrocarbon chain and polar head molecular structure contributes greatly to our understanding of monolayer elasticity at a microscopic level.

3   Elastic Parameters of Mixed Monolayers

The existing theoretical approaches describing monolayers composed of different kinds of amphiphile molecules are based on the original theories (1) and (2), where the elastic moduli κ, $\bar{\kappa}$ and the spontaneous curvature c_0 are determined by the elastic parameters of the individual components. According to the thermodynamical analysis [18], the

dependence of the elastic parameters of a monolayer on its composition can have a complicated character. In particular, it has been demonstrated for monolayers of mixtures of diblock copolymers consisting of molecules with different block lengths [19]. In a simpler model the spontaneous and intrinsic curvatures are supposed to be averages of the characteristics of the components. For example, for a two-component monolayer consisting of amphiphiles with intrinsic curvatures c_{in}^1, c_{in}^2, the resulting spontaneous curvature is modeled as

$$c_{in} = \theta c_{in}^1 + (1 - \theta)c_{in}^2 \tag{3}$$

where θ is the molar fraction of the first component in the mixed system (varying from 0 to 1).

Only recently [20, 21] the experimental studies allowed direct verification of this model. Measurements of structural parameters of H_{II} phases composed of two phospholipids DOPE and dioleoylphosphatidylcholine (DOPC) [20] indicate that the intrinsic curvature is a linear function of their relative composition. Further confirmation of the linear relation (3) over a wide range of compositions is found in another recent study of H_{II} phases consisting of DOPE and dioleoylphosphatidylglycerol (DOG) [21].

The situation is less clear for the bending modulus of mixed amphiphilic membranes. Models of monolayers of diblock copolymers predict complex nonlinear dependence on the composition [19]. Similar results follow from the mean-field approach [17]. Furthermore, the experimental determination of the bending elasticity of monolayers composed of DOPE and DOG [21] demonstrates nonlinear dependence of κ on the composition. Therefore, modeling of the bending elasticity of mixed membranes consisting of lipids and detergents still poses a challenge and more refined theories are needed.

Another related issue concerns the value of the Gaussian curvature of mixed amphiphilic membranes and has been addressed only in few studies. Mean-field theories [17, 22] computed numerically the change of $\bar{\kappa}$ as function of the ratio of two amphiphiles having different length of hydrophobic chain. Analytical expression for $\bar{\kappa}$ has been derived only for mixed monolayers of diblock copolymers [19]. However, we do not know of any experimental investigations which directly attempted to obtain $\bar{\kappa}$ of mixed membranes.

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This question remains open for both theoretical and experimental studies.

4   Theoretical Models of Mixed Aggregates

Attempts have been made to interpret all types of aggregates observed in experiments on mixed amphiphiles. The specific questions addressed in these studies are: (i) the existence of stable single-component lipid vesicles characterized by a preferred size; (ii) the increase of vesicle sizes upon addition of small amounts of micelle-forming surfactants; (iii) the transition of mixed vesicles into mixed micelles at a critical lipid-to-surfactant ratio, and, finally, (iv) the preferred shapes of mixed micelles.

Thermodynamic stability of single-component lipid vesicles cannot be understood only in terms of model (1) [23]. Indeed, a *symmetric* lipid bilayer has a vanishing spontaneous curvature. Therefore, the bending energy of a spherical vesicle is equal to $F = 4\pi(2\kappa + \bar{\kappa})$ (neglecting corrections of order δ/R, δ and R are the membrane thickness and vesicle radius, respectively) and does not depend on its radius. The bending modulus κ is positive but the Gaussian curvature modulus $\bar{\kappa}$ can have negative values. Depending on the relationship between κ and $\bar{\kappa}$ the vesicle energy F can be either positive or negative, and vesicles in a suspension have, respectively, a tendency to grow or to decrease their size. To understand the existence of a preferable radius of one-component vesicles, one has to account, in addition to the bending energy (1), for other factors like the translational entropy of vesicles in a dilute water solution or the effects of interactions between vesicles in dense suspensions.

Stability of mixed vesicles can be understood by considering the possibility of a spontaneous curvature of bilayers resulting from asymmetric partitioning of different amphiphiles between the two membrane leaflets [23]. Analysis of the bending energy of such systems took into account the entropy of mixing in the membrane and an additional interaction between the amphiphiles in the bilayer. The model [23] provided an explanation of experiments by Kaler et al [24] on formation of stable vesicles for mixtures of cationic and anionic surfactants.

Nevertheless, the growth of vesicle size upon addition of micelle-forming surfactants is

still not very well understood since simple models predict an opposite behavior; namely, a decrease in the vesicle size due to added surfactants. Indeed, the concentration of surfactant molecules should be higher in the outer leaflet (as compared with the inner one) of a spherical vesicle since the curvature there has the same sign as the spontaneous curvature of the micelle-forming surfactant. It results in an increase of the spontaneous curvature of the bilayer favoring formation of vesicles of smaller radii.

The addition of surfactant is also affecting the Gaussian curvature modulus of the bilayer $\bar{\kappa}$. Insertion of micelle-forming molecules in membrane monolayers is known [25] to change $\bar{\kappa}$ of the bilayer towards more negative values. It should also enhance the tendency of lipid vesicles to separate into smaller aggregates rather than to grow in size. These considerations may indicate a possible reason for the decrease of vesicle size as observed very recently in systems of dimeric amphiphiles mixed with surfactants [9]. On the other hand, the more common observation of the increase in the vesicle size remains unexplained.

5   Conclusions

Theoretical descriptions of phase transition of mixed liposomes into mixed micelles has been formulated using the elastic model (2)-(3) [26] and a more microscopic mean-field approach accounting for the amphiphile chain packing [27]. Besides the bending energy, the entropy of mixing of the two components has been shown to determine the phase behavior of the system. The predicted phase diagram of mixed amphiphiles has all the qualitative features of the experimental data. An assumption of the model, based on the experimental observations, was that the micelles have (in the coexistence region) the shape of infinitely long cylinders. On the other hand, other aggregate and micellar forms have been suggested in the literature. The spheroidal micelles [9] and the perforated bilayers [10] have been directly observed, while the disc-like micelles remain a hypothesis used at earlier stages to explain the results of dynamic light scattering. Analysis of the factors determining the shapes of amphiphilic aggregates resulting from solubilization of liposomes by micelle-forming surfactants is the subject of much current interest. Hope-

fully, further theory and experiments in this area will resolve some of the outstanding issues.

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